THE ISOELECTRIC POINT OF WOOL

By Milton Harris [1]

Suspensions of solvent-extracted Idaho and Australian raw wool and of scoured worsted cloth in buffer solutions of different pH were prepared by grinding the dry wool to a fine powder and shaking the powder in the buffer solution. Electrophoretic measurements of these suspensions gave an isoelectric point for each wool at pH 3.4.

The samples were slightly different in nitrogen content. If this is indicative of a difference in structure of the wool, the results indicate that the isoelectric point is very slightly or not at all affected by small differences in constitution.

The theory and application of electrophoresis measurements to suspensions of wool are discussed.

CONTENTS

I. INTRODUCTION

The application of physical chemical methods to studies of wool fiber should make it possible to understand better the complex character of this protein as well as offering a means of determining any changes taking place during chemical processing.

Since wool is amphoteric; that is, is capable of ionizing both as an acid and a base, it follows that a study of its acidic and basic nature is of practical as well as theoretical importance. Amphoteric substances are positively charged in acid solution and negatively charged in alkaline solution. The hydrogen ion concentration of a solution in which the ionization of the acid groups of the amphoteric substances, ampholytes, is equal to the ionization of the basic groups is known as the isoelectric point. That is, at this point the concentrations of the cations of the ampholyte equals the concentration of the anions and the sum of their concentrations is a minimum.

A study of the reactions of the wool protein in the isoelectric region is of importance at this time, since more emphasis is being placed on utilization of pH control in wool processing and also because the values of the isoelectric point found by other workers are not in agreement.

It has been shown that it is necessary to control the pH in the treatment of wool during manufacturing processes. Solomone [2] has

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pointed out that there are optimum pH values for the scouring, oiling, bleaching, and dyeing of wool. A specific example is brought out in the chlorination of wool. If this operation is carried out in the isoelectric region, the attack on the fibers is reduced to a minimum.

The isoelectric point has been reported as low as pH 3.4 and as high as pH 4.8. Meunier and Rey[3] determined the point of minimum swelling of wool fibers immersed in buffer solutions of varying pH, and found the isoelectric point at pH 3.6 to pH 3.8. Marston[4] investigated the amount of acid bound in the region of the isoelectric point. By extrapolating the curve relating the amount of acid bound with the pH of the reacting system, he found that the point at which no acid is bound lies in the region of pH 3.4. Speakman[5] treated samples of wool with standard potassium ferrocyanide solutions of known pH. After 24 hours the wool was removed and carefully washed to remove uncombined ferrocyanide and then immersed in ferric chloride solution. By this procedure he found that when the pH was high no combination with the ferrocyanide ion occurred; at pH 4.8, combination began and thereafter the amount of Prussian
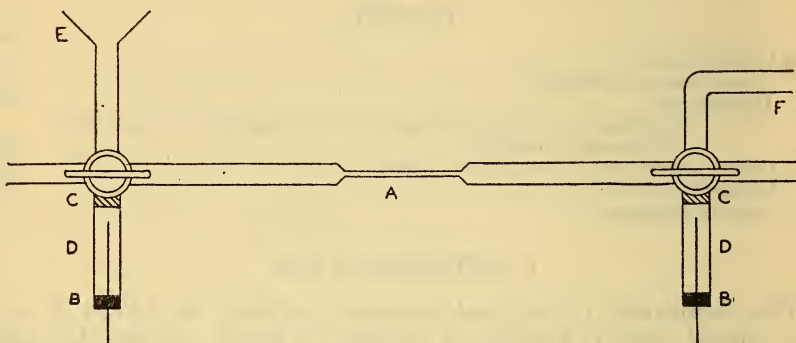

FIGURE 1.—*Electrophoresis cell*

blue formed increased as the pH decreased. He concluded from this that the isoelectric point of wool is in the neighborhood of pH 4.8.

A number of methods are available for the determination of the isoelectric points of proteins, but the applicability of any specific method depends largely on the nature of the protein in question. These methods are based on the fact that at the isoelectric point, the osmotic pressure, viscosity, amount of alcohol required for precipitation, conductivity, swelling, and migration in an electric field are all a minimum.

The insolubility of wool in ordinary solvents has limited the number of these methods which can be used in studying this material. Since microscopic suspensions of wool are easily prepared, it is possible to study the migrations of the particles in an electric field. The movement of small particles suspended in a liquid in which a potential difference between two electrodes has been set up is known as electrophoresis.

[3] Meunier and Rey, "Sur les Propriétés de la laine," J. Soc. Leather Trades Chemists, vol. 11, p. 508, 1927.
[4] Marston, the Chemical Composition of Wool, Council Sci. & Ind. Res. Australia, Bull., vol. 38, 1928.
[5] J. B. Speakman, the Behaviour of Wool as an Amphoteric Colloid, J. Soc. Dyers and Colorists, vol. 41, p. 172, 1925.

II. APPARATUS AND METHODS

The electrophoresis cell (fig. 1) employed in this work is similar to the one described by Northrup[6] and is exactly like the one used by Abramson.[7] *A* is made of fused glass with approximate dimensions as follows: Length 3.5 cm, width 1 cm, and thickness 0.055 cm. The diameter of the connecting side tubes is large in comparison with the thickness of the cell. The electrodes, *D D*, are nonpolarizable and composed of $Cu/CuSO_4$. *C C* are porous plugs prepared as follows: Powdered gypsum is put into the electrode vessels, sufficient water added, and the mixture is allowed to dry and harden for several hours. The apparatus is filled with saturated potassium chloride solution and allowed to stand until the pores of the plaster of Paris plugs are saturated with electrolyte.

The stopcocks are turned so that *A* is open to *E* and *F*. The suspension to be studied is poured into *E* and both stopcocks turned so that *A* is open to *D D*. The apparatus is placed on the platform of a microscope and a direct current of 220 volts is applied to the electrodes. The current is reversed by means of a commutator. The microscope is equipped with an 8 mm objective and a 6X eyepiece. The eyepiece contains a scale which covers 0.34 mm on the stage micrometer.

The electrophoretic velocity (see Sec. III) was measured in each direction and an average of 10 readings was taken.

In the determination of the isoelectric point, three samples of wool were used, namely, raw Idaho wool, raw Australian wool, and wool from a piece of worsted cloth which had not been carbonized. The wool grease and dirt were removed from the raw wool by successive extractions with Stoddard solvent (boiling range 150° to 200° C), alcohol, and ether until no appreciable residue was obtained on evaporation of the solvent. The worsted cloth was scoured in a half per cent soap solution after which it was carefully washed with water, alcohol, and ether to remove any traces of soap and oils remaining.

The purified wool was ground in a Wiley mill until the powder formed passed through a 100-mesh sieve. The powder was shaken with water and the larger particles allowed to settle to the bottom after which the upper portion was decanted. The finer particles remained suspended in the liquid for several hours.

The buffers were prepared according to Clark[8] and the final pH determined by employing the quinhydrone electrode. The buffer mixtures used were as follows: pH 1.4 to 2.2, potassium chloride-hydrochloric acid mixture; pH 2.4 to 3.8, acid potassium phthalate-hydrochloric acid mixtures; pH 4.0 to 6.0, acid potassium phthalate-sodium hydroxide mixtures.

III. THEORETICAL

When the movement of suspended particles is observed in successive layers from top to bottom of a cell as has just been described, it will be seen that particles in the layer next to the glass move toward the cathode as if they were positively charged. By observing layers from the glass toward the center of the cell, a layer will be found where

[6] J. Northrup, The Stability of Bacterial Suspensions, J. Gen. Physiol., vol. 4, p. 629, 1922.
[7] H. Abramson and H. Freundlich, Uber die Kataphoretische Wanderings-geschwindigkeit gröbere Teilchen in Solen und Gelen, Z. Phys. Chem., vol. 128, pp. 25–38, 1927.
[8] W. M. Clark, The Determination of Hydrogen Ions Williams and Wilkins, pp. 106–107.

there is no apparent movement. Below this point, the direction of migration on the particles reverses and their velocity increases until the center is reached.

Since there is a movement of the liquid with respect to the glass wall of the cell as the result of an applied electromotive force, it follows that the liquid must be oppositely charged with respect to the wall. Apparently a double electric layer exists at the interface of liquid and wall, the water being positive and the glass surface negatively charged.

The movement of liquids and suspended particles in flat electrophorsesis cells has been investigated by numerous workers. Ellis[9] assumed that for a flat cell of depth X

$$Vobs = V_p + V_w$$

where

$Vobs =$ The observed electrophoretic velocity of the particle.
$V_p =$ The velocity of the particle relative to the liquid.
$V_w =$ the velocity of the liquid.
In the layer where $V_w = 0$, $Vobs = V_p$.

In his theoretical considerations Von Smoluchowski[10] has shown that the layers at which the velocity of the liquid is zero are located at approximately one-fifth and four-fifths of the depth of the cell from the top. That is

$$V_p = V_{1/5} = V_{4/5} = 1/x \int_0^X Vobs\ dx$$

where x is the depth in the cell. He also showed that the velocity of a particle in either layer is equal to three-fourths of the observed velocity of a particle in a layer one-sixth of the depth from the top plus one-fourth of the observed velocity of a particle in a layer at one-half of the depth.

Experimental work of Ellis and of Svedberg and Anderson[11] have confirmed this theory for flat cells from 50 μ to about 1.0 mm in thickness.

In order to determine that the cell used conformed to this theory Vobs was measured at successive levels in the cell and V_p was obtained by graphical integration. Crude quartz powder was purified as described by Abramson,[12] and suspended in distilled water. The movement of the suspended particles in the electrophoresis cell is shown in Figure 2. The shape of the curves depends on the pH of the solution. By graphical integration it was found that the theory of Von Smoluchowski was applicable to the cell used in this work.

[9] R. Ellis, Die Eigenschaften der Olemulsionen, Z. Physik., Chem., vol. 78, p. 321, 1912.
[10] M. Von Smoluchowski in Gratz Handbuch der Electrizität und des Magnestisms, vol. 2, p. 366, 1921.
[11] T. Svedberg and H. Anderson, Zur Messmethodik der Electrischen Kataphorese, Kolloid Z, vol. 24, pp. 156–165.
[12] H. Abramson, Electrokinetic Phenomena, J. Gen. Physiol, vol. 13, p. 171, 1929.

1. THE INFLUENCE OF SIZE AND SHAPE OF WOOL PARTICLES ON THEIR ELECTROPHORETIC MOBILITY

The electrophoretic velocity V of a particle relative to a given medium is

$$V = \frac{1}{4\pi} \cdot \frac{XD\zeta}{n} \tag{4}$$

Where

X = field strength.
D = dielectric constant of the liquid.
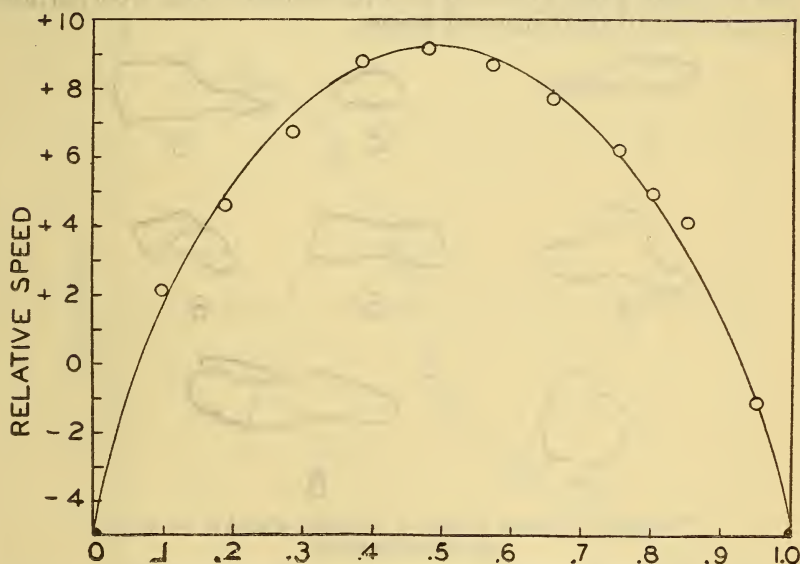ζ = electro-kinetic potential.
n = viscosity of the liquid.

FIGURE 2.—*Velocity—level curve for the electrophoresis cell used in the experimental work*

This relationship was first developed by Helmholtz [13] and later extended by Perrin and Von Smoluchowski. [14]

This equation predicts that the electrophoretic velocity is independent of the size and shape of the particle. Abramson [15] has summarized experiments which confirm this. The experiments indicate that the electrophoretic mobility of solid particles like quartz, glass particles, asbestos needles, and tyrosine needles is independent of size and shape in the size ranges indicated.

A sample of wool prepared as previously described was suspended in a dilute buffer solution at approximately pH 4.8. The particles varied in size from about 5 to 30 μ. The time required for different sizes and shapes of particles to move across the field was measured. The general outline of the shape and the relative size of each of the

[13] H. Helmholtz, Studien uber Electrische Grenzschichten, + Wied Ann, vol. 7, 337–383, 1879.
[14] See footnote 9, p. —.
[15] H. Abramson, Influence of Size, Shape, and Conductivity on Cataphoretic Mobility, J. Phys. Chem., vol. 35, pp. 289–308, 1931.

particles noted is shown in Figure 3 while the corresponding time required to cross the scale is given in Table 1.

TABLE 1.—*Relative speed of wool particles*

Particle	Time
	Seconds
1	14.1
2	14.3
3	13.6
4	13.9
5	14.1
6	13.6
7	13.9
8	13.5

The results in Table 1 indicate that the mobility of the wool particles is independent of their size and shape.
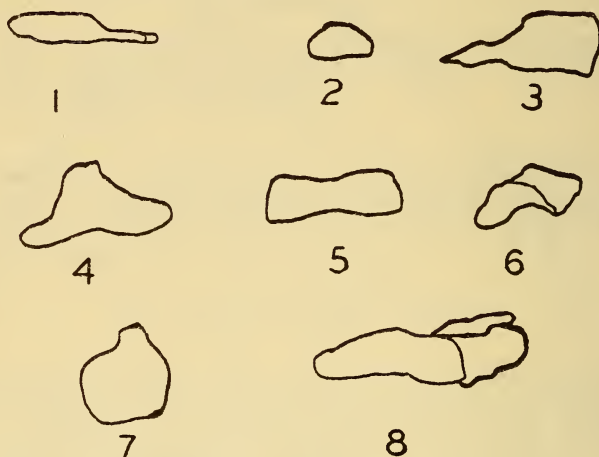
FIGURE 3.—*General outline of the shape of each of the various particles noted*

They are drawn to approximately the same scale. (Two dimensions.)

IV. DETERMINATION OF THE ISOELECTRIC POINT

Suspensions of wool in buffer solutions of known pH were prepared as described in an earlier part of this paper and the electrophoretic velocities of the wool particles determined.

TABLE 2

pH	μ/sec./volt/cm				Average percentage deviation from mean
	Idaho wool	Australian wool	Worsted cloth	Average velocity	
1.54	+2.50 +2.30 +2.38	+2.44 +2.50 +2.38	+2.32	+2.40	2.91
1.93	+2.20 +2.22 +2.21	+2.29 +2.27 +2.18	+2.16	+2.22	1.70
2.30	+2.08 +2.03 +2.04	+2.16 +2.08 +2.16	+2.04	+2.08	2.11
2.52	+1.93 +1.86 +1.90	+1.93 +1.96 +1.93	+1.89	+1.93	1.36
2.91	+1.32 +1.24 +1.23	+1.40 +1.28 +1.38	+1.31	+1.32	3.92
3.31	+.48 +.46 +.47	+.52 +.50 +.52	+.54	+.50	6.26
3.48	−.54 −.61 −.58	−.55 −.51 −.61	−.62	−.58	4.84
3.64	−.94 −.89 −1.00	−1.03 −.98 −.99	−1.03	−.98	3.98
4.11	−1.42 −1.25 −1.39	−1.50 −1.46 −1.45	−1.42	−1.41	3.56
4.63	−1.86 −1.90 −1.95	−1.90 −1.98 −1.94	−1.98	−1.94	1.94
5.47	−2.61 −2.50 −2.46	−2.46 −2.51 −2.48	−2.45	−2.52	1.74
Average					3.12

The observed velocities are given in Table 2 in which each figure is the average of 10 observations. These data are plotted in Figure 4 which shows the shape of the pH mobility curve. The value assigned to the isoelectric point from this curve is pH 3.4.

The accuracy of this value depends upon the accuracy of the measurements of the pH and of the velocity of the wool particles. The error in the determination of the velocities, although comparatively large, is practically negligible because of the steep slope of the curve in the isoelectric region. Since the pH is known to be accurate to
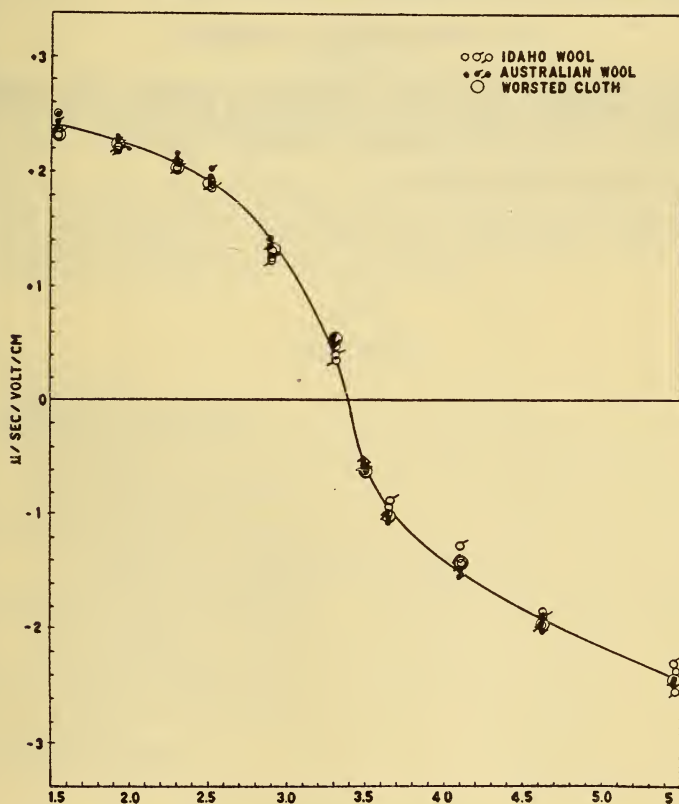

FIGURE 4.—*pH mobility cure for raw Idaho and Australian wool and for worsted cloth*

± 0.05, this may be taken as the limiting error for the isoelectric point of wool.

V. DISCUSSION OF RESULTS

The isoelectric point 3.4 obtained from the pH mobility curve is in good agreement with the value 3.4 obtained by Marston [16] and the value 3.6–3.8 obtained by Meunier and Rey.[17] The results of the individual measurements on the raw Idaho and Australian wool and the worsted cloth are also in good agreement with each other.

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[16] See footnote 4, p. 780.
[17] See footnote 3, p. 780.

Analyses of the samples for total nitrogen gave the following values:

	Per cent nitrogen
Australian wool	16. 19
Idaho wool	16. 48
Worsted cloth	17. 16

Assuming that this variation in nitrogen content is some indication of a variation in structure, the results of the electrophoretic measurements confirm the theory that the isoelectric point is only slightly or not at all affected by small differences in structure.

VI. ACKNOWLEDGMENT

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